

Control of damping-off and root rot diseases of *Acacia* tree using some biological control agents and plant extracts

FATIMA HADI KAREEM*

*Al-Mussaib Technical College
Al-Furat Al-Awsat Technical University, 51009, Babylon, Iraq
(e-mail : com.fatma@atu.edu.iq)*

(Received : November 21, 2019/Accepted : December 28, 2019)

ABSTRACT

The study included the isolation and diagnosis of the associated fungi to damping-off and the root rot disease of the *Acacia* plants and evaluating the effectiveness of some biological agents in controlling the disease *in vitro*. The results of isolation and diagnosis from the infected roots of *Acacia* plant showed the presence of the two pathogenic fungi (*Rhizoctonia solani* and *Fusarium solani*), which appeared frequently in all samples brought from some nurseries in Babylon province. The isolates of two pathogenic fungi (*R. solani* and *F. solani*) affected the germination of *Acacia* seeds which led to occurring a significant exelling through raising the intensity of the infection with two pathogenic fungi (*R. solani* and *F. solani*) which amounted to 83.33 and 41.66%, respectively, compared to the control treatment, in which the intensity of the infection amounted to zero. The two fungi had also significantly reduced the dry and fresh weights of the total vegetative and root system. The results of the antagonistic potential test for the fungus (*Trichoderma harzianum*) showed high efficiency in inhibiting the two pathogenic fungi, which achieved an antagonistic potential on the PSA media. *Azospirillum* sp. bacteria showed high antagonistic potential in inhibiting the two pathogenic fungi (*R. solani* and *F. solani*) with a percentage of 64.1 and 61.1, respectively. The aqueous extract for the plants (fruits of castor, leaves, and flowers of *Clerodendrum inerme*, garlic oil) showed an effective effect in inhibiting the pathogenic fungi on the PSA. The extract of the castor fruit plant gave better efficiency than rest of the extracts where the percentage of inhibition to the two pathogenic fungi for the aqueous extract for the castor plant at a concentration of 15% amounted to 56.66 and 54.81%, respectively.

Key words : *Acacia*, biological control, damping-off, fungi, plant extracts, root rot disease

INTRODUCTION

Acacia tree belongs to the Fabaceae family. Asia and West Africa are considered the original home for this tree. It has an importance, where the oils are extracted from it in addition to the dyes. Some perfume is extracted from its flowers, its wood is famous for its aromatic aroma, their flower is rich in nectar and is considered an important pasture for bees. The honey of *Acacia* flowers is considered one of the finest honey. However, in Iraq, Beekeepers do not care about it due to the lack of sufficient areas cultivated with it. It has medicinal uses, where the leaves are boiled to treat chest and lung pain. The roots are used to treat teeth and stomach pains (Hayward, 2004).

Acacia tree is subjected to infection with many fungal pathogens. These diseases

include damping-off and the root rot (Dar *et al.*, 2018). This disease causes many endemic fungi in the soil and the intensity of the infection with these fungi is associated with increase and decrease in temperature and soil moisture (Webster and Weber, 2007; Lahuf *et al.*, 2019). In order to reduce the impact of these causes, low-cost and easy-to-use programs have been used, such as the use of microorganisms to reduce the pathogenic vaccine, with increasing the production of both qualitatively and quantitatively, which is characterized by biological control such as the use of *Trichoderma harzianum* and *Azospirillum* sp. (Verma *et al.*, 2007; Ulker *et al.*, 2011), where *T. harzianum* and *Azospirillum* sp. have shown high efficiency against various soil fungi such as *Rhizoctonia solani* and *Fusarium solani* (Akrami and Yousefi, 2015; Al-Hammouri *et al.*, 2018).

These programs also include the use of plant extracts which differ in their chemical properties and therefore differ in their effect on pathogenic organisms such as fungi and bacteria, where their high specialization, rapid degradation and non-pollution of the environment were among the most prominent characteristics that encouraged their use (Hantoosh, 2016). There are no studies on fungus causing the disease of damping-off and the root rot of the *Acacia* tree in Iraq. The study aims at isolating the fungi associated with the roots of *Acacia* tree infected with the disease and evaluating the effectiveness of the *T. harzianum* fungus and *Azospirillum* sp. bacteria in the growth of pathogenic fungi that cause disease in the *Acacia* tree and evaluating the effectiveness of the aqueous extract for the plants (fruits of castor, leaves and flowers of *Clerodendrum inerme*, garlic oil) in the growth of some pathogenic fungi that cause disease in the *Acacia* tree.

MATERIALS AND METHODS

Isolation and Diagnosis of Fungi

Samples for the roots of the *Acacia* plants that cultivated inside the greenhouses were taken from Babylon province on November 23, 2015, which showed the symptoms of infection as yellowing of the leaves and ulceration or rot of the stem base and root rot. The roots were taken to the laboratory in polyethylene sacs and washed with running water for 15 min to remove the soil from it. The roots were then cut into small pieces (1-0.5 cm) and then sterilized by immersing them with sodium hypochlorite solution at 1% concentration for 3 min. The sterilized roots were then washed with sterile distilled water for 2 min and were transferred to sterilized filter paper for the purpose of removing excess water and then transferred by sterile forceps to Petri dish with a diameter of 9 cm. containing an culture media (Potato Sucrose Agar PSA) (200 g potato, 10 g sucrose, 20 g agar, 1 l of distilled water) sterilized at a temperature of 121°C and pressure of (1.5 kg/cm²) for 20 min with autoclave and tetracycline at concentration of (250 mg/l) with rate of four pieces Petri dish. The dishes were incubated for three days at a temperature of 25±2°C. The dishes were then examined and the different

fungi were purified. They were examined by the Compound Microscope. The species were identified based on approved taxonomic keys (Parmeter and Whitney, 1970; Seifert, 1996; Blazier and Conway, 2004; Leslie and Summerell, 2006).

Antagonistic Potential Test

Effect of two pathogenic fungi (*R. solani* and *F. solani*) in *Acacia* plants : This test was conducted in the lath house belonging to the Technical Institute/Al-Mussaib in at 4-2-2016. Where paper pots were used with a capacity of 1 kg of soil, the pots were filled with sterile soil at 121°C and pressure of 1.5 kg/cm² for 1 h. The following day was sterilized for an hour, left for seven days, the soil was then inoculated with *R. solani* and *F. solani*. The two fungal vaccines were added by 1% (weight/weight) loaded on the local millets seeds. Each treatment was repeated three times with three replicates by adding sterile millet seeds only as a control, the pots were wetted with water and after three days of inoculating the soil with the fungal vaccine, the pots were cultured with *Acacia* seeds, with rate of five seeds for each pot, the pots were irrigated after culturing and the percentage for the intensity of infection after 30 days of culture was calculated using the following pathological index :

0. White total vegetative and the root hairs intact
1. Colouring 1-25% of the root with light brown colour
2. Discolouration of more than 25-50% of the root in dark brown colour
3. Colouring more than 50-75% of the root with dark brown colour with falling lower leaves
4. Colouring more than 75-100% of the root with dark colour and plant death

The intensity of the infection was calculated according to the following equation (McKinney, 1923) :

$$\text{Intensity of the infection} = \frac{(\text{Number of plants in degree } 0 \times 0) + (\text{Number of plants in degree } 1 \times 1) + (\text{Number of plants in degree } 4 \times 4)}{\text{Number of tested plants} \times 4} \times 100$$

Testing the antagonistic potential of *Trichoderma harzianum* against two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media :

The antagonistic potential of *T. harzianum* was tested which was obtained from the Department of Biotechnology, Al-Mussaib Technical College, Postgraduate Studies using double culture technique. The sterilized culture media PSA was prepared as previously mentioned and the media was distributed with a 9-cm diameter Petri dish. The dishes were then left until the culture media was hardened and then vaccinated by taking a 0.5 cm disc by the sterilized cork borer near the edges of the fungus colony (*R. solani* and *F. solani*) each individually, which grew on the PSA media with the age of 3 and 7 days, respectively. The disc was placed in the center of half the dish and the center of half of the other dish has been vaccinated with a disk diameter of 0.5 cm taken by the sterilized cork borer near the edges of the colony of biological control fungi which grew on the PSA media at the age of seven days, Three dishes were used for each treatment. The dishes were placed in the incubator at 25±2°C for seven days and the antagonistic was estimated according to the Bell scale and the other (1982), consisting of five degrees as follows :

1. Anti-fungus covers all the dishes with the pathogen.
2. Anti-fungus covers two-thirds of the dish.
3. Anti-fungus covers half the dish.
4. Pathogenic fungus covers two-thirds of the dish.
5. Pathogenic fungus covers all the dishes.

The antifungal is effective if the antagonistic degree between 1 and 2.

Testing the antagonistic potential of *Azospirillum* sp. against two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media :

The isolation of *Azospirillum* sp. bacteria was obtained from the Department of Biotechnology, Al-Mussaib Technical College, Postgraduate Studies. They were propagated on the nutrient agar media, where the media was sterilized in the autoclave without using the antibiotic. After that, they were propagated on nutrient broth in a 100 ml flask and placed in the autoclave for 20

min, then left to cool. Three tablets were taken from the nutrient agar media on which the bacteria were grown and placed in the flask containing the liquid media, then placed in the incubator at 30±2°C for three days (Ballah *et al.*, 2015). The antagonistic potential of *Azospirillum* sp. against two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media was tested by adding 1 ml of the grown bacteria on the media (NB) in the dish with moving it in a circular motion to distribute the bacterial vaccine in a homogenous manner. The center of each dish was then inoculated with a disk, its diameter of 0.5 cm that was taken from near the edges of the colony of two pathogenic fungi grown on the PSA media with three days age for the fungus *R. solani* and seven days for fungus *F. solani*. Three dishes were used for treatment and three dishes were left without adding bacteria as a control (Singh *et al.*, 2015). The dishes were incubated at a temperature of 25±2° for seven days and the amount of inhibition or the percentage of inhibitory was then calculated by calculating the diameter of the grown fungi colony in the bacteria treatment and comparing it with the diameter of the grown fungi colony in the control treatment. The percentage of inhibition of fungal growth was calculated according to the following equation (Montealagre *et al.*, 2003) :

Percentage of inhibition = 1- (fungal growth in bacterial treatment/ fungal growth in the control treatment) × 100

Plant extracts : Three plants were selected to study their effect against two pathogenic fungi (*R. solani* and *F. solani*) including castor, garlic and *C. inerme* (Table 1).

Plant collection and sample preparation : The plants were collected at the beginning of the flowering. As for the garlic plant, they were obtained from the local market and the plant samples were dried under the sun by spreading them in the form of thin layers on the surfaces of the carton and flipping them constantly to accelerate drying. After drying, the plant samples were grinded using an electric mill and the power of each plant was placed in polyethylene bags and kept in a dry place until use.

Table 1. Plants used in the study

Local name	Scientific name	Plant family	The used part	The collected area
Castor	<i>Ricinus communis</i> L.	Euphorbiaceae	Fruits	Al-Mussaib gardens
Garlic	<i>Allium sativum</i>	Amaryllidaceae	Oil	Local markets
<i>C. inerme</i>	<i>Clerodendrum inerme</i> L.	Verbenaceae	Leaves and flowers	House garden

Preparation of the aqueous extract :

Seema *et al.* (2011) method was followed in the preparation of the aqueous extracts by mixing 20 g of dry plant powder for each plant sample separately with 400 ml of distilled water in a 1000 ml glass flask. The suspension was then left in a water bath at 30°C for half an hour and then spraying the suspension using several layers of gauze and it was then sterilized through the millipore filter; the diameter of their holes 0.22 ppm. The liquid was kept in closed containers in the refrigerator and left for use.

Testing the effect of plant extracts for castor, garlic, *C. inerme* in the growth of the two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media : The method of Al. Quraishi (2011) was followed by mixing the aqueous extract of the selected plants with the soluble PSA media after sterilization and coiling for 45°C. Take 5, 10 and 15 ml of the extract and add to 85, 90 and 95 ml, respectively, from the PSA media at the rate of three replicates per concentration. After hardening the culture media, the dishes were then inoculated in a center with a 0.5 cm disc from the edge of a fungal colony growing on the PSA media for both the pathogenic fungi (*R. solani* and *F. solani*) at the age of 3 and 7 days, respectively. The dishes were incubated at a temperature of 25±2° and after the arrival of the diameter of fungal culture for the control treatment (without extract) to the edge of the dish. The results were calculated by calculating the average of two perpendicular diameters from the growth of each colony. The percentage of inhibition was calculated using the following equation :

$$\text{Inhibition (\%)} = \frac{\text{The average of fungus growth in the control treatment} - \text{The average of fungus growth in the treatment}}{\text{The average of fungus growth in the control treatment}} \times 100$$

RESULTS AND DISCUSSION**Isolation and Diagnosis**

The results of isolating the fungus from the infected plant parts showed for the roots of Acacia plants and the nearby stem regions from the samples that were collected from some nurseries (Al-Musayyib and Al-Hilla/Babylon region) which showed symptoms of plant weakness. The appearance of ulcers on the stems, rotting the roots with brown colour and presence of the two pathogenic fungi (*R. solani* and *F. solani*). It is a soil fungus that has a pathogenic importance to many plant families, causing different symptoms of the disease, including root rot (Mishra, 2017). From another hand, *R. solani* and *F. solani* are highly resistant to difficult environmental conditions, where *R. solani* is characterized by its formation of stone bodies that enable it to remain for long periods of time in the soil. *F. solani* is characterized by the formation of chlamydospores, which have the ability to remain in orbit for at least five years and sometimes 10 years (Li *et al.*, 1998).

Testing the Antagonistic Potential

Effect of the two pathogenic fungi (*R. solani* and *F. solani*) in Acacia plants : Table 2 shows that the isolate of the two pathogenic fungi (*R. solani* and *F. solani*) led to occurring a significant increase in the intensity of infection with the two pathogenic fungi compared to the control treatment, in which the intensity of infection is zero, while in the fungus treatment (*R. solani* and *F. solani*) amounted to 83.33 and 41.66%, respectively. The reason for the ability of the pathogenic fungus *R. solani* to bring such proportions due to its various mechanisms known such as secretion of enzymes for the analysis of host cells or the secretion of metabolic substances with toxic effect, which leads to the failure of germination (Mohammed *et al.*, 2006). Baker

Table 2. Effect of the two pathogenic fungi (*R. solani* and *F. solani*) in the percentage for the intensity of infection and some growth parameters for *Acacia* plants

Treatment	The percentage of infection*	The percentage for the intensity of infection	Fresh weight (g)		Dry weight (g)	
			Total vegetative	Root system	Total vegetative	Root system
<i>R. solani</i>	100	83.33	0.73	0.31	0.07	0.05
<i>F. solani</i>	60	41.66	1.00	0.69	0.35	0.06
Control	0	0	2.48	1.82	0.43	0.13
LSD (P=0.05)	23.07	28.64	0.28	0.46	0.47	0.03

*Each figure in table represents an average for three replicates.

et al. (1981) and Nelson *et al.* (1997) reported that *F. solani* secreted a number of toxins, including Fusarubin, Javanicin, Anhydro fusarubin, Protenoneous, and Polypeptide, which play an important role in fungal pathogens, or possibly due to the enzymatic activity of fungi, where it produces a number of proteolytic enzymes including Pectolytic, Cutinase and Celluloytic Enzymes (Vidhyasekaran, 1997). Table 2 shows that the isolates of the two pathogenic fungi led to a significant reduction in the measured growth parameters compared to the control treatment. The average weight of the total vegetative and root system for the infected plants with an isolate of *R. solani* amounted to 0.73 and 0.31 g, respectively, *F. solani* (1 and 0.69 g), respectively, compared to the control treatment which their average for the total vegetative and root system amounted to 2.48 and 1.82 g, respectively. The isolate of the two pathogenic fungi caused a significant reduction in the dry weight of the total vegetative and root system which their average amounted to 0.07, 0.05, 0.35 and 0.06 g, respectively, compared to the control treatment which the averages of their total vegetative and root system amounted to 0.43 and 0.13 g, respectively.

Testing the antagonistic potential of *Trichoderma harzianum* against two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media : The results of the antagonistic experiment between *T. harzianum* and the isolate of the two pathogenic fungi (*R. solani* and *F. solani*) showed that the fungus had a high antagonistic potential towards the two pathogenic fungi, where achieved an antagonistic amounted to 1 and 1, respectively, according to Bell *et al.* (1982). This was due to the fact that *T. harzianum* had several mechanisms such as the production of toxic

metabolic materials for fungal pathogens or the production of many fragmented enzymes for fungal cell walls or may be due to direct intrusions by spinning the mycelium for the biotic around hypha pathogens (Harman, 2006; Kareem and Matloob, 2019).

Testing the antagonistic potential of *Azospirillum* sp. against two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media : The results showed the ability of *Azospirillum* sp. bacteria to inhibit the growth of two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media. The average growth of the two pathogenic fungi (*R. solani* and *F. solani*) amounted to 3.23 and 3.5 cm, respectively, compared to the control treatment which amounted to 9.00 and 9.00 cm, respectively. The average percentage for inhibition of the two pathogenic fungi amounted to 64.1 and 61.1%, respectively, as shown in Table 3. This was due to the ability of bacteria to secretion auxins, most notably indole acetic acid (IAA), as well as their secretion to plant hormones such as gibberellins and cytokinins (Ryu *et al.*, 2003). As well as the production of low-molecular-weight compounds that are resistant to pathogenic fungi, including the hydrogen

Table 3. Testing the antagonistic potential of *Azospirillum* sp. against two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media

Treatment	The average growth of fungus* (cm)	The inhibition
<i>R. solani</i> + <i>Azospirillum</i> sp.	3.23	64.10
<i>F. solani</i> + <i>Azospirillum</i> sp.	3.50	61.10
<i>R. solani</i>	9.00	0.00
<i>F. solani</i>	9.00	0.00
LSD (P=0.05)	0.64	7.12

*Each figure in table represents an average for three replicates.

cyanide compound, where the presence of this compound at high concentrations inhibits the growth of pathogenic fungi. This result is similar to that of Singh *et al.*, (2015) that the bacteria had inhibited the growth of the fungus *R. solani* on the PDA media with the percentage of 55, which causes the disease of root rot of wheat.

Testing the effect of plant extracts for castor, garlic, *Clerodendron inerme* in the growth of the two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media :

The results in Table 4 show that there were differences in the effect of aqueous extract for castor, garlic oil, *C. inerme* depending on plant type, the concentration of extract and tested fungus. The results of the statistical analysis showed significant differences at the level of 5% between the average growth of the colonies of the two pathogenic fungi (*R. solani* and *F. solani*) in different treatments. It was found that the aqueous extract for the fruits of the castor plant caused the highest percentage of inhabiting in the growth of two pathogenic fungi, where gave a percentage of inhabiting amounted to 56.66 and 54.81%, respectively, at the concentration of 15% compared to the control treatment (without extract). At the concentrations of 5 and 10%, the percentage of inhabiting of the castor plant to the two

pathogenic fungi (*R. solani* and *F. solani*) amounted to 5.55, 39.25, 11.11 and 41.48%, respectively. The inhibiting efficiency for the aqueous extract of the castor plant is attributed to the presence of some alkaloids, such as Riciuine, N-dimethyl ricinine and O-ricinine-dimethyl. In addition to ricin oil, which is toxic and important in the inhibition process as well as containing alkaloids and fatty acids (Oleic and linolic acid) and phenols and resins and contains the substance of saponins, which help in the inhibition process (Swaati *et al.*, 2014). These results agree with those of Mahmood *et al.* (2014) in their study by using some plant extracts, including the castor extract, which their using led to inhibit the growth of *F. solani* fungus after several days of using on the culture media. The extract of garlic oil, *C. inerme* gave an inhibition to the growth of the two pathogenic fungi (*R. solani* and *F. solani*) where the percentage of inhibiting for the garlic oil extract at the concentration of 15% against the two pathogenic fungi amounted to 43.7 and 46.29%, respectively, the percentage of inhibiting for the *C. inerme* extract at the same concentration against the two pathogenic fungi amounted to 29.25 and 32.95%, respectively. Sadda and Rashmi (2015) showed that the extract of castor and garlic was efficient in controlling the root rot disease caused by the *R. solani* fungus, where it was found that the

Table 4. Effect of aqueous extracts activity for castor, garlic oil, *Clerodendron inerme* plants in inhibiting the two pathogenic fungi (*R. solani* and *F. solani*) on PSA culture media

Treatment	Concentration	The average growth of fungus* (cm)	The inhibition
<i>R. solani</i> +Castor	5	8.50	5.55
<i>R. solani</i> +Castor	10	5.46	39.25
<i>R. solani</i> +Castor	15	3.90	56.66
<i>F. solani</i> +Castor	5	8.00	11.11
<i>F. solani</i> +Castor	10	5.26	41.48
<i>F. solani</i> +Castor	15	4.06	54.81
<i>R. solani</i> +Garlic	5	9.00	0.00
<i>R. solani</i> +Garlic	10	6.03	32.95
<i>R. solani</i> +Garlic	15	5.06	43.70
<i>F. solani</i> +Garlic	5	8.66	3.70
<i>F. solani</i> +Garlic	10	6.00	33.32
<i>F. solani</i> +Garlic	15	4.83	46.29
<i>R. solani</i> + <i>C. inerme</i>	5	9.00	0.00
<i>R. solani</i> + <i>C. inerme</i>	10	6.71	25.36
<i>R. solani</i> + <i>C. inerme</i>	15	6.36	29.25
<i>F. solani</i> + <i>C. inerme</i>	5	9.00	0.00
<i>F. solani</i> + <i>C. inerme</i>	10	6.26	30.36
<i>F. solani</i> + <i>C. inerme</i>	15	6.03	32.95
<i>R. solani</i>	0	9.00	0.00
<i>F. solani</i>	0	9.00	0.00
LSD (P=0.05)		0.32	0.59

*Each figure in table represents an average for three replicates.

extract of castor and garlic gave the best results in the control of *R. solani* fungus, therefore they were the best alternatives than chemical pesticides because they are environmentally safe, inexpensive, non-hazardous and did not disturb the balance of the environment. As for the *C. inermis* extract, Aparna and Girija (2018) found that the most important terpenes compounds in the *C. inermis* plant were 3-epicaryoptine, B-clerodendrin, 15-hydroxy epicary optine, clerodendrin, it was found that it had an inhibiting activity to the growth of the organism and with low concentrations.

CONCLUSION

The pathogens for the disease of damping-off and the root rot for the *Acacia* tree are the fungi (*Rhizoctonia solani* and *Fusarium solani*). And the efficiency of the aqueous extract for the castor fruits in inhibiting the growth of the two pathogenic fungi (*R. solani* and *F. solani*) *in vitro*. The biological control agent *T. harzianum* and *Azospirillum* sp. showed high antagonistic potential in inhibiting the growth of pathogenic fungi on the PSA media.

REFERENCES

- Akrami, M. and Yousefi, Z. (2015). Biological control of *Fusarium* wilt of tomato (*Solanum lycopersicum*) by *Trichoderma* spp. as antagonist fungi. *Biological Forum* **7** : 887-92.
- Al-Hammouri, A. A., Lindemaan, W., Thomas, S. and Sanogo, S. (2018). Effect of inoculum levels of *Rhizoctonia solani* and *Meloidogyne incognita* on chile pepper in soil simultaneously infested with both the pathogens. *Res. Crops* **19** : 509-19.
- Al-Quraishi, M. K. F. (2011). Evaluating the effectiveness of some plant extracts in the growth of some pathogenic fungi. Master thesis, College of Science, Karbala University, Iraq.
- Aparna, K. P. and Girija, V. K. (2018). Effect of biofumigation with plant extracts on mycelial growth and sclerotial germination of *Rhizoctonia solani* causing collar rot and web blight of cowpea. *Int. J. Curr. Microbiol. App. Sci.* **7** : 2990-99.
- Baker, R. A., Tatum, J. H. and Nemeč, S. (1981). Toxin production by *Fusarium solani* from fibrous roots of diseased citrus. *Phytopathology* **71** : 951-54.
- Ballah, N. T., Akshmi, P. M. and Akshmi, V. R. (2015). Effect of *Azospirillum* on the growth and biochemical characters of okra [*Abelmoschus esculentus* (L.) Moench]. *Int. J. Adv. Res.* **3** : 1272-80.
- Bell, D. K., Wells, H. D. and Markham, C. R. (1982). *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology* **72** : 379-82.
- Blazier, S. R. and Conway, K. E. (2004). Characterization of *Rhizoctonia solani* isolates associated with patch disease on turf grass. *Proc. Oklahoma Acad. Sci.* **84** : 41-51.
- Dar, W. A., Hassan, M. G., Sheikh, P. A., Summuna, B. and Ganaie, S. A. (2018). Integrated disease management capsule for wilt/root rot complex of chili. *Int. J. Curr. Microbiol. App. Sci.* **7** : 1253-61.
- Hantoosh, M. N. K. (2016). Activity of cumin and cinnamon extract in controlling fungus *Rhizoctonia solani* the cause to damping-off cotton seedling. *Euphrates J. Agric. Sci.* **8** : 222-28.
- Harman, G. E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* **96** : 190-94.
- Hayward, B. (2004). The acacia tree Asusta-Projramme NR International Ltd. Park House Bradbourne Lane, Aylesford. Kent ME20.6 SNUK.
- Jafar, O. H. (2011). Biological and chemical control of cowpea wilting disease caused by *Rhizoctonia solani* Kuhn and *Fusarium solani* (Mart) Sacc. Master thesis, Al-Furat Al-Awsat Technical University, Al-Mussaib Technical College, Iraq.
- Kareem, F. H. and Matloob, A. A. H. (2019). Efficiency of some biological control agents and plant extracts against *Fusarium solani* causing agent of damping off disease on tomato. *Plant Archives* **19** (Supplement 2) : 937-42.
- Lahuf, A. A., Alfarttoosi, H. A., Al-Sweedi, T. M. and Middlefell-Williams, J. E. (2019). Evaluation of an integration between the nano-sized zinc oxide and two cultivars for the control of damping off disease in sunflower crop. *Res. Crops* **20** : 174-79.
- Leslie, J. F. and Summerell, B. A. (2006). *The Fusarium Laboratory Manual*. pp. 388.
- Li, S. X., Hartman, G. L. and Gray, L. E. (1998). Chlamyospore formation production and nuclear status in *Fusarium solani* f. sp. glycines soybean sudden death syndrome causing isolates. *Mycologia* **90** : 414-21.
- Mahmood, R., Memona, N., Ahmad, A. S., Anam, U. and Answer, A. (2014). Screening of indigenous weed extracts against *Fusarium solani* with an emphasis on soil fertility related microbial activities. *J. Food Agric. Environ.* **12** : 958-62.

- McKinney, H. H. (1923). Influence of soil temperature and moisture on infection of wheat seedling by *Helminthosporum sativum*. *J. Agric. Res.* **26** : 195-217.
- Mishra, P. K. (2017). Study on fungi. *Pak. J. Biol. Sci.* **4** : 17-19.
- Mohamed, I. A. I., Bauomy, M. A. M. and Ibrahim, A. S. A. (2006). Efficacy of different natural products as safe management of guar damping off disease in Egypt. *Egypt J. Phytopathol.* **34** : 115.
- Montealagre, J. R., Reyes, R., Perez, L. M., Herrera, R., Silva, P. and Besoalin, X. (2003). Selection of bioantagonistic bacteria to be used in biological control *Rhizoctonia solani* in tomato. *Electronic J. Biotechnol.* **6** : doi : 10.2225/vol6-issue2-fulltext-8.
- Nelson, B. D., Hansen, J. M. and Helms, T. C. (1997). The reaction of soybean cultivars to isolates of *Fusarium solani* from the Red River valley. *Plant Dis.* **81**: 664-68.
- Parmeter, J. R. and Whitney, H. S. (1970). Taxonomy and nomenclature of the imperfect stage. In : *Rhizoctonia solani Biology and Pathology*, J. R. Parmeter (ed.). University of California, Barkely, Los Angeles. pp.7-19.
- Ryu, C. M., Faray, M. A. and Hu, C. H. (2003). Bacterial volatiles promote growth in arabidopsis. *Proc. National Academy of Sciences (LISA)* **100** : 4927-32.
- Sadda, N. and Rashmi, V. (2015). Bioefficacy of plant extracts in the control of root rot disease of sponge gourd. *J. Indian Bot. Soc.* **94** : 126-30.
- Seema, M., Sreenivas, S. S., Rekha, N. D. and Devaki, N. S. (2011). *In vitro* studies of some plant extracts against *Rhizoctonia solani* Kuhn infecting FCV tobacco in Karnataka light soil, Karnataka. *Int. J. Agric. Technol.* **7** : 1321-29.
- Seifert, K. (1996). Fuskey, *Fusarium* interactive key. Agriculture and Agri-food, Canada.
- Singh, P., Prashant, S. and Singh, M. P. (2015). Assessment of antifungal activity of PGPR (Plant growth promoting rhizobacterial) isolates against *Rhizoctonia solani* in wheat (*Triticum aestivum* L.). *Int. J. Adv. Res.* **3** : 803-12.
- Swaati, S., Verma, N. and Garg, V. (2014). Screening of phytochemical constituents of hydro-ethanolic extracts of aerial parts of *Pithecellobium dulce* and *Ricinus communis*. *Res. J. Chem. Sci.* **4** : 54-57.
- Ulker, S., Ozel, A., Colak, A. and Karaoglu, S. A. (2011). Isolation, production and characterization of an extracellular lipase from *Trichoderma harzianum* isolated from soil. *Turk. J. Biol.* **35** : 1-8.
- Verma, M., Brar, S. K., Surampalli, R. Y. and Valero, J. R. (2007). Antagonistic fungi *Trichoderma* spp. Panoply of biological control. *Biochem. Engg. J.* **37** : 1-20.
- Vidhyasekaran, P. (1997). Fungal pathogenesis in plants and crops. Molecular biology and host defense mechanism. Marcel Dekker, INC. pp. 542.
- Webster, J. and Weber, R. W. S. (2007). *Introduction to Fungi, 3rd edn*. Published in the United States Press, New York. pp. 875.